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# Histone Hyperacetylation in the Coding Region of Chromatin Undergoing Transcription in SV40 Minichromosomes Is a Dynamic Process Regulated Directly by the Presence of RNA Polymerase II

**Lata Balakrishnan and Barry Milavetz***

*Department of Biochemistry and Molecular Biology, University of North Dakota, Grand Forks ND 58203, USA*

SV40 chromosomes undergoing transcription operationally defined by the presence of RNA polymerase II (RNAPII) were immune-selected with antibody to RNAPII and subjected to secondary chromatin immunoprecipitation with antibodies to hyperacetylated or unacetylated H4 or H3. Immune selection fragmentation and immunoprecipitation was used to determine the hyperacetylation status of histones independent of the location of the RNAPII and Re chromatin immunoprecipitation was used to determine their hyperacetylation status when associated with RNAPII. While hyperacetylated H4 and H3 were found in the coding regions regardless of the location of RNAPII, unacetylated H4 and H3 were found only at sites lacking RNAPII. The absence of unacetylated H4 and H3 at sites containing RNAPII was correlated with the specific association of the histone acetyl transferase p300 with the RNAPII. In contrast, the presence of unacetylated H4 and H3 at sites lacking RNAPII was shown to result from the action of a histone deacetylase based upon the effects of the inhibitor sodium butyrate. These results suggest that the extent of hyperacetylation of H4 and H3 during transcription alternates between hyperacetylation directed by an RNAPII associated histone acetyl transferase and deacetylation directed by a histone deacetylase at other sites.



*Corresponding author

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## Introduction

The histones that are organized with eukaryotic DNA to form chromatin undergo diverse forms of post-translational modifications.[1] Because these modifications can potentially impact the interactions between the histones and associated DNA or other proteins, there has been great interest in understanding the function of these modifications in eukaryotic biological processes. The covalent addition of acetyl groups to histone tails is one form of modification that has been investigated extensively and has been associated with transcription in a number of different studies.[2,3] Moreover, the enzymes responsible for histone acetylation and deacetylation, histone acetyl transferases (HATs) and histone deacetylases (HDACs), respectively, have been studied extensively,[4] and in many cases the HATs and HDACs have been found to be associated with various transcription factors.[5]

These studies, while clearly establishing a link between histone hyperacetylation and transcription, are limited by the fact that invariably they are based upon an association between a difference in the extent of transcription such as the induction of a gene and the changes in the properties of the total cellular chromatin containing that gene. To date, there has not been an extensive characterization directly of histone hyperacetylation in chromatin that is specifically undergoing transcription *in vivo*.

We have been using the simian virus 40 (SV40) chromosome as a eukaryotic model to investigate two aspects of chromatin structure, nucleosome phasing and histone hyperacetylation.[6–9] The SV40

Abbreviations used: HAT, histone acetyl transferase; HDAC, histone deacetylase; RNAPII, RNA polymerase II; ChIP, chromatin immunoprecipitation; ReChIP, Re chromatin immunoprecipitation; ISF, immune selection and fragmentation; ISFIP, immune selection fragmentation and immunoprecipitation.

E-mail address of the corresponding author: bmilavetz@medicine.nodak.edu

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chromosome is particularly well suited for this function, since it utilizes host cell proteins for its transcription and replication, and has been studied extensively as a model for both eukaryotic processes.[10–13] In the SV40 model system, transcription has been shown to occur in three distinct phases (Figure 1(a)). Within approximately 1 h after infection, the first phase of transcription occurs with the induction of the early region of the SV40 genome. The second phase of transcription occurs at about 8 h post-infection when the virally encoded early protein T-antigen interacts with its cognate binding site in the promoter and down-regulates further early transcription. The third phase of transcription, which occurs either along with or shortly after down-regulation of early transcription, is characterized by a marked increase in transcription from the late side of the genome.[10]

In order to obtain transcribing SV40 chromosomes for direct analysis of histone hyperacetylation, we reasoned that by using an antibody specific to RNA polymerase II (RNAPII), a protein that is absolutely required for transcription, we would be able to specifically immune-select transcribing SV40 chromosomes using chromatin immunoprecipitation (ChIP) procedures.[14–16] We expected that the immune-selected SV40 chromatin would consist of chromosomes with RNAPII located in the promoter, which were undergoing initiation, chromosomes with RNAPII located in coding regions, which were undergoing transcriptional elongation, and chromosomes with RNAPII located in either the promoter or coding regions in which the RNAPII was paused. We confirmed that antibody to RNAPII could be used to immune-select transcribing SV40 chromosomes using a modified ChIP procedure that we refer to as immune selection and fragmentation (ISF).[6,7] In the ISF procedure, the immune-selected transcribing SV40 chromosomes were fragmented by sonication into bound chromatin fragments that

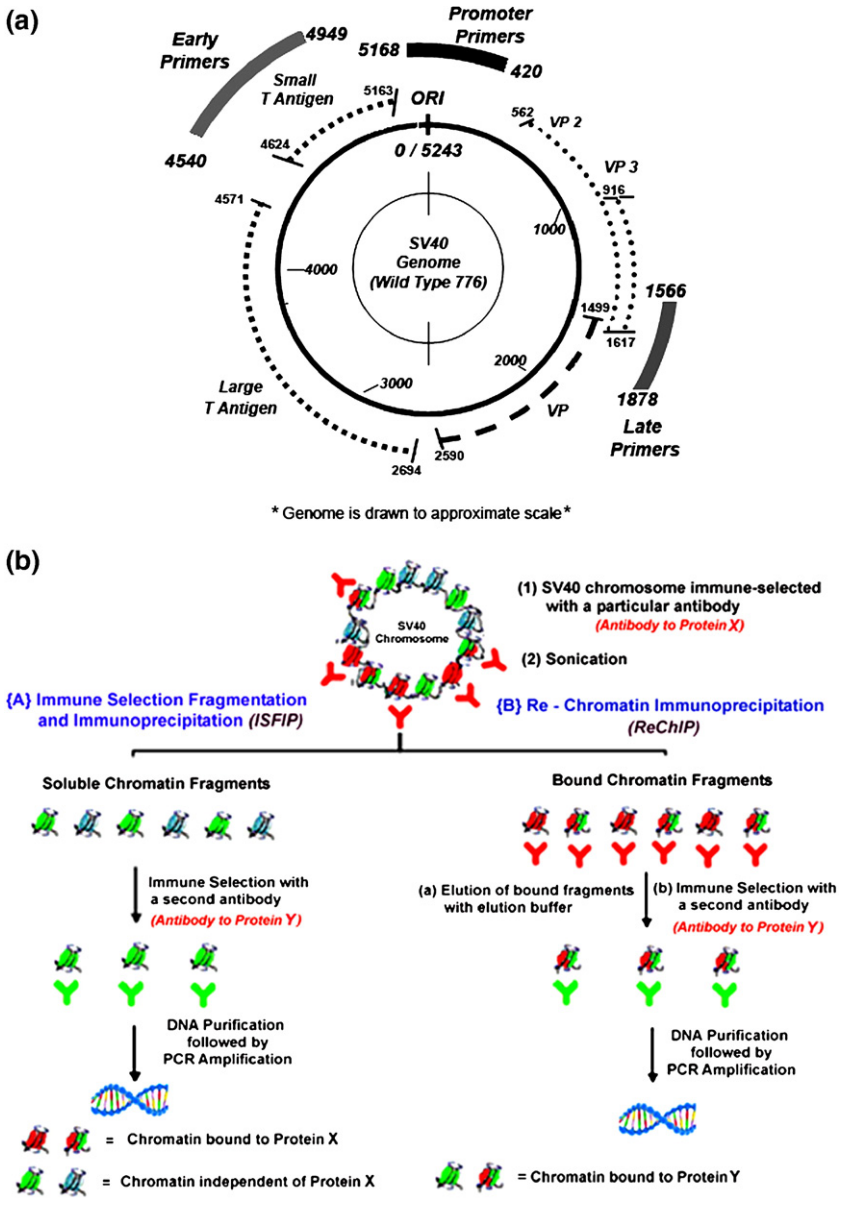


**Figure 1.** (a) The SV40 genome. (b) Strategy of immune selection fragmentation followed by immunoprecipitation (ISFIP) and Rechromatin immunoprecipitation (ReChIP).